E (ApoE) promoter, HCR-1, HCR-2, the AI apolipoprotein liver-specific enhancer and the a1-antitrypsin promoter.

69. The pharmaceutical composition of claim 67, wherein said therapeutic protein is factor VIII and said therapeutic effect is an improvement in the coagulation defect.

70. The pharmaceutical composition of claim 67, wherein said therapeutic protein is factor IX and said therapeutic effect is an improvement in the coagulation defect.--

REMARKS

I. The above amendments place the instant application in condition for allowance, or at the very least, simplify issues for consideration and appeal. A regulatory region for gene expression in human liver cells is supported by the teachings on page 24, lines 15-21; and at page 25, line 25 through page 26, line 2 of the instant specification. Delivery to the portal vasculature of a mammal is taught at page 31, lines 3-8 of the instant specification. The particular therapeutic proteins of interest, factor VIII, factor IX and GM-CSF, are taught in the instant specification at page 19, lines 12-13 as well as at page 21, lines 31-34. A diffusible protein is taught in the instant specification at page 11, lines 13-18. A tissue-specific promoter active in hepatic cells is taught at page 25, lines 5-9 of the instant specification. Particular regulatory regions for gene expressions in human liver are taught at page 25, line 25 through page 26, line 2 of the instant specification. The effect of factor VIII on improvement of a coagulation defect is taught at page 10, lines 5-18 of the instant specification.

Hence, no issue of new matter arises with the above-presented amendments and the new claims, and thus, entry thereof is requested respectfully.

II. Turning now to the Office Action, on page 2, the Examiner noted that the claim for priority was lacking in the specification.

Applicants thank the Examiner for the reminder and have complied by the above-noted amendment to the specification.

III. At the top of page 3 of the Office Action, the Examiner raised an issue with respect to the nomenclature of a vector.

Again Applicants thank the Examiner and have used a consistent descriptive name as provided by the above amendments.

IV. Also on page 3 of the Office Action, the Examiner raised an objection to claim 4 as not limiting claim 1.

Without capitulating to the position of the Examiner, it is believed that amended claim 4 properly limits the scope of amended claim 1. Therefore, the objection can be removed.

V. At the top of page 4, claims 1, 4, 7, 15, 16, 20, 26, 27, 31, 32, 36, 39 and 43 were rejected under 35 U.S.C. § 112, first paragraph for an alleged want of enablement.

The rejection is traversed for the following reasons.

The use of AAV for gene delivery in a therapeutic context is well established in the art. Numerous clinical trials using AAV to deliver genes and to express desired genes are underway. The instant specification teaches in vivo models demonstrating proper expression of a therapeutic protein of interest.

A post filing date declaration setting forth test results substantiating utility "pertains to the accuracy of a statement already in the specification. It does not render an insufficient disclosure enabling, but instead goes to prove that the disclosure was in fact enabling when filed." In re Brana, 34 USPQ2d 1436 (Fed. Cir.1995).

Applicants can offer testimony based on a publication as evidence of the level of skill in the art at the time the application was filed, see Gould v. Quigg, 822 F.2d 1074, 1077, 3 U.S.P.Q.2d 1302, 1304 (Fed. Cir. 1987). Accordingly, Applicants submit that the accuracy and enablement of the disclosure is supported by the following references, copies of the references are attached hereto.

Wang L. et al. (Mol. Ther. 2000 Mar. 1(2):154-158) describe studies wherein a single intraportal vein injection of a recombinant adeno-associated virus (rAAV) vector encoding canine factor IX (cFIX) cDNA under the control of a liver-specific enhancer/promoter led to a long-term correction of the bleeding disorder in hemophilia B dogs.

Chuah M.K. et al. (J. Gene Med. 2001 Jan-Feb. 3(1):3-20) describe the development of gene therapy for the treatment of hemophilia A and B, where therapeutic and physiologic levels of FVIII and FIX were achieved in FVIII-deficient and FIX-deficient mice, and in hemophiliac dogs using different gene therapy approaches. The reference states that long-term correction of the bleeding disorders and in some cases a permanent cure was realized in some preclinical studies and that several gene therapy clinical trials are currently ongoing in patients suffering from severe hemophilia A or B.

Kay MA et al. (Nat. Genet. 2000 Mar. 24(3):257-261) describe a clinical trial where an AAV vector expressing human FIX was administered to adults with severe hemophilia B, based on the results of pre-clinical studies in mice and hemophiliac dogs. The results show

gene transfer and expression of factor IX following intramuscular injection of an AAV vector expressing human FIX.

Chao H et al. (Blood 2000 Mar. 95(5):1594-9) describe sustained expression of hFVIII in immunocompetent mice using rAAV vectors. The results of in vivo experiments showed expression of hFVIII in the plasma following portal vein injection.

Liu Y et al. (J. Interferon Cytokine Res. 2000 Jan. 20(1):21-30) describe the transduction of dendritic cells (DC) with an AAV/GM-CSF/Neo vector, followed by detection of GM-CSF secretion, GM-CSF RNA expression (by RT-PCR) and cell proliferation. The authors suggest that AAV-mediated transduction of GM-CSF may replace the need for exogenous GM-CSF in the production of mature DC.

Koeberl D et al. (Human Gene Ther. 2000 Sept. 10:2133-2140) describe systemic administration of rAAV that expressed GM-CSF. Neutrophil numbers increased in the presence of the expressed transgene. Expression lasted at least five months.

Accordingly, a prima facie case of non-enablement has not been made. The instant specification and the claimed invention are in full compliance with 35 U.S.C. § 112, first paragraph. Hence, the rejection can be removed.

VI. On page 8, claims 15, 31 and 32 were rejected under 35 U.S.C. § 112, second paragraph.

The rejection is traversed for the following reasons.

The rejection is moot with respect to claim 15. As to claims 31 and 32, the claims have been amended to recite that the regulatory elements are liver-specific.

In view thereof, withdrawal of the rejection is in order.

VII. At the top of page 9 of the Office Action, claims 1, 16 and 26 were rejected under 35 U.S.C. § 102(a) over Fisher et al.

Fisher et al. describe rAAV vectors that include viral ITRs and a CMV promoter driven galactosidase gene. Accordingly, Fisher et al. do not teach each and every element of claims 1, 16 and 26 as provided hereinabove. For example, Fisher et al. do not teach a therapeutic gene or a liver-specific regulatory region.

Hence, the rejection is improper and must be removed.

VIII. At the bottom of page 9 of the Office Action, claims 1 and 26 were rejected under 35 U.S.C. § 102(b) over the Ponnazhagan et al. Blood abstract.

Ponnazhagan et al. describe AAV vectors containing a CMV promoter driven galactosidase gene and β globin under control of the endogenous promoter. The Blood abstract does not teach each and every element of the claimed invention. For example, the Blood abstract does not teach a therapeutic gene or a liver-specific regulatory region.

Accordingly, anticipation does not lie and the rejection must be removed.

IX. At the top of page 10 of the Office Action, claims 1 and 26 were rejected under 35 U.S.C. § 102(b) over Koeberl et al.

The rejection is traversed for the following reasons.

Koeberl et al. describe an AAV vector containing the MLV LTR promoter driven galactosidase gene and an AAV vector with hFIX under control of the LTR promoter. Tests were conducted in vitro in rat dermal fibroblasts. Koeberl et al. do not teach each and every

element of the claimed invention. For example, neither liver-specific expression, a therapeutic gene nor a liver-specific regulatory region is taught therein.

Accordingly, anticipation does not exist and the rejection can be removed.

X. At the bottom of page 10 of the Office Action, claims 1, 4, 7 and 26 were rejected under 35 U.S.C. § 102(e) over Chiorini et al., U.S. Pat. No. 5,693,531.

The rejection is traversed for the following reasons.

The '531 patent describes a dual vector AAV system wherein the second vector, which includes DNA sequences encoding AAV rep and cap proteins, has an inducible origin of replication, e.g., the SV40 origin of replication, which is activated the SV40 T-antigen. The '531 patent does not teach each and every element of the claimed invention. For example, the '531 patent does not teach a liver-specific regulatory region.

Hence, the rejection is improper and must be removed.

XI. Finally, on page 11 of the Office Action, claims 1, 7, 15, 16, 20, 26 and 43 were rejected under 35 U.S.C. § 102(e) over Wilson et al., U.S. Pat. No. 5,756,283.

The rejection is traversed for the following reasons.

The '283 patent does not teach each and every element of the invention as claimed. For example, the '283 patent does not teach a liver-specific regulatory region.

Accordingly, there is no anticipation and the rejection can be removed.

CONCLUSION

Applicants have taken steps to place the instant application in condition for allowance. In view thereof, withdrawal of the rejections and early indication of allowance are solicited earnestly. If any questions remain unresolved, the Examiner is urged to contact the undersigned at the local exchange noted hereinbelow.

The Commissioner hereby is authorized to charge or to credit any shortage or surplus to Deposit Account No. 18-2220.

Respectfully submitted,

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Dated: 28 February 2002

ADENO-ASSOCIATED VIRAL VECTOR-MEDIATED DELIVERY OF DNA TO CELLS OF THE LIVER

The instant application ...

ACKNOWLEDGEMENTS

The invention disclosed in the instant application was supported in part by NIH grant HL53682. The U.S. Government may have rights in certain portions of the invention disclosed herein.

INTRODUCTION

BACKGROUND

The first human gene therapy trial started in September of 1990 and involved retrovirally-mediated transfer of the adenosine deaminase (ADA) gene into lymphocytes of patients with severe combined immunodeficiency (SCID). The favorable results of that trial stimulated further interest in gene therapy resulting in additional gene therapy clinical protocols approved by the NIH Recombinant DNA Advisory Committee (RAC). Although the original promise of gene therapy was the development of a curative treatment for simple, single gene diseases, the vast majority of gene therapy trials were directed to complex genetic or acquired diseases, such as cancer and infectious disease. A large number of the initial clinical gene transfer studies were not gene therapy per se but rather gene marking studies. The first type of marking experiments used tumor infiltrating lymphocytes which were transduced in vitro with retroviral vectors prior to infusion into patients with cancer. The second class of gene marking studies involved the attempt to detect residual tumor cells in marrow infused into patients following ablative chemotherapy.

Of the currently approved gene therapy trials, all trials prior to 1992 used retroviral vectors and the diseases targeted included SCID, familial hypercholesteremia and cancer. More recently, gene therapy trials have commenced for AIDS and Hemophilia B, again using retroviral

Fig. 5 depicts the location and orientation of binding of the D1 and D2 primers, which can distinguish wild-type AAV from those generated by recombination between the vector and helper plasmids.

Fig. 6 is a schematic representation of the rAAV-MFG-human-Factor IX vector. ITR = AAV Inverted Terminal Repeat. MFG Promoter = Murine Moloney Virus Long Terminal Repeat. MuLV IVS = mRNA splice donor/splice acceptor. Human FIX = human Factor IX gene. bGH pA = bovine growth hormone polyadenylation site.

Fig. 7 is a schematic diagram outlining the relationship of the AAV helper plasmid, AAV vector plasmid, adenovirus helper virus and 293 cells for the production of rAAV vectors.

Fig. 8 is a graph showing high-level expression of human Factor IX in immunocompetent mice that were injected intraportally with 2.1×10^{10}, 4.2×10^{10} and 8.4×10^{10} particles of rAAV-MFG-hFactor IX.

Fig. 9 is a graph showing expression of human Factor IX in immunocompromised mice that were injected intraportally with 4.2×10^{10} particles of rAAV-MFG-hFactor IX.

Fig. 10 is a graph showing expression of human Factor IX in immunocompetent mice that were injected intraportally with 4.2×10^{10} particles of rAAV-MFG-hFactor IX in combination with additional treatments.

Fig. 11 is a diagram of the SSV9-MFG-S huFIX vector plasmid.

DESCRIPTION OF SPECIFIC EMBODIMENTS

LOCAL AND SYSTEMIC DELIVERY OF THERAPEUTIC AMOUNTS OF RECOMBINANT PROTEINS FROM THE LIVER

The instant invention demonstrates that therapeutic levels of a protein or polypeptide can be achieved locally and systemically by delivering to the liver of a mammal a recombinant AAV vector carrying an exogenous or endogenous polynucleotide encoding that protein or polypeptide. Not

exclusively within hepatocytes. At one week, there were rare fields with any immunostained cells. However by weeks 3 and 5, positive hepatocytes were found in most fields; multiple groups of immunostained hepatocytes were found around the portal vasculature while many of the immunostained cells were scattered randomly throughout the parenchyma. The TH positive hepatocytes at three and five weeks represented a small percentage of the hepatocyte population. The low number could indicate a high level of gene expression from a relatively few transduced cells or result for elimination of some of the transduced cells by an immune response.

To further establish the percentage of transduced cells in mice receiving rAAV-hFIX, in situ hybridization (described in greater detail hereinbelow) for hFIX mRNA's in two mice at 11 weeks had about 2 to 5% positive hepatocytes in a distribution similar to that seen for the mice receiving rAAV-TH. About 5% of the 10^8 hepatocytes in a mouse liver were assumed to express factor IX and that on average the liver contains 2 AAV genomes per diploid cell, then there are 40 genome copies per transduced cell. The alternative is that there are some cells which contain rAAV genomes that do not express transgene product.

Infusion of recombinant adenovirus vectors into the portal vasculature results in an early and more chronic pattern of low level liver injury as determined by liver enzyme elevation and inflammatory cell infiltrates (Lieber et al., J. Virol., 70:8944-8960 (1996)). Interestingly, the toxicity may not result from an immune response directed at the viral particle but rather to viral antigens whose genes are present in the vector (Lieber et al. (1996)).

To establish whether similar toxicity occurs with rAAV, 5 animals injected with 4.2×10^{10} rAAV-MFG-~~hFIX~~ human Factor IX particles were monitored for serum pyruvic glutamic transaminase (SGPT), a sensitive serum marker for hepatic injury. The SGPT concentrations were all in the normal range during the first nine days and similar to that found in a naive control

mouse. Similarly, there was no evidence of histological infiltrates in the liver during the first week and at seven weeks after AAV administration.

EXAMPLE 6. DETECTION OF FACTOR IX EXPRESSION IN IMMUNOCOMPETENT MICE BY WESTERN BLOT ANALYSIS.

~~Recombinant~~rAAV-MFG-Human Factor IX is delivered to the liver as a single intraportal administration of $4.2x10^{10}$ particles in a volume of 200 microliters of Dulbecco's Modified Eagle's Medium (DMEM) to a C57BL/6 mouse. The mouse is bled at three weeks after administration. The blood sample is diluted and is subjected to Western blot analysis using human serum as a standard. The blot is probed using polyclonal antibodies to human Factor IX.

Expression of human Factor IX was observed as a protein of about 70 kb.

EXAMPLE 7. FACTOR IX EXPRESSION IN IMMUNOCOMPETENT MICE USING RAAV VECTOR IN COMBINATION WITH OTHER TREATMENTS.

~~Recombinant~~ rAAV-MFG-Human Factor IX is delivered to the liver as a single intraportal administration of $4.2x10^{10}$ particles in a volume of 200μl of Dulbecco's Modified Eagle's Medium (DMEM) to C57BL/6 mice. In addition to the rAAV, some mice can receive in the same administration either adenovirus Ad.DE2hAAT-D or adenovirus AdRSVhAAT (Lieber et al., J. Virol., 70:8944-8960 (1996)), or the rAAV can be administered alone to a mouse receiving a partial hepatectomy prior to infusion. Mice are bled at the indicated number of weeks after administration depicted in Figure 5, and the blood samples are stored frozen. The expression of human Factor IX is determined by ELISA assay as in Example 4.

EXAMPLE 8. MAINTENANCE OF THE RAAV VECTOR IN MOUSE LIVER

Following post intraportal infusion of the rAAV-MFG-Human Factor IX, DNA can be isolated from liver and spleen from two rAAV-treated mice. DNA is also isolated

WHAT IS CLAIMED IS:

1. (Amended) A method of expressing a polynucleotide in a mammal, comprising:

administering viral particles comprising a recombinant adeno-associated virus (AAV) vector to liver cells of said mammal, wherein said recombinant AAV (rAAV) vector comprises said polynucleotide, such that said polynucleotide, or a portion thereof, is expressed in said mammal.

2. The method of claim 1, wherein said polynucleotide is expressed in vivo.

3. The method of claim 2, wherein said mammal is a human.

4. (Amended) The method of claim 1, wherein liver cells of said mammal are transduced with said ~~recombinant~~ rAAV ~~vector~~ ex vivo, and wherein said administering further comprises delivering said transduced liver cells into the portal vasculature of said mammal.

5. The method of claim 4, wherein said mammal is a human.

6. The method of claim 5, wherein said polynucleotide encodes a therapeutically effective polypeptide for treating a human disease or disorder.

7. (Amended) The method of claim 1, wherein said polynucleotide of said rAAV vector comprises:

a promoter capable of expression in human liver cells,

a structural gene encoding a therapeutically effective polypeptide, and

two (AAV) Inverted Terminal Repeats, wherein said Inverted Terminal Repeats flank the promoter and structural gene.

8. The method of claim 7, wherein said Inverted Terminal

Repeats comprise a portion of said wild-type AAV Inverted Terminal Repeats.

9. The method of claim 7, wherein said promoter is obtained from a virus.

10. The method of claim 9, wherein said virus is murine leukemia virus.

11. The method of claim 10, wherein said virus is Moloney murine leukemia virus.

12. The method of claim 11, wherein said rAAV vector is rAAV-MFG-human Factor IX.

13. The method of claim 4, wherein said polynucleotide comprises an antisense polynucleotide.

14. The method of claim 4, wherein said polynucleotide encodes a ribozyme.

15. (Amended) The method of claim 1, wherein said therapeutic protein is diffusible further comprising:
providing said mammal with a partial hepatectomy.

16. The method of claim 1, further comprising:
administering a helper virus into the portal vasculature of said mammal.

17. The method of claim 1, further comprising:
administering a secondary agent for enhancing transduction efficiency to said liver cells of said mammal.

18. The method of claim 17, wherein said administering of said secondary agent and said administering of said recombinant AAV vector occurs in vivo.

19. The method of claim 3, wherein a secondary agent is

applied to said liver cells of said mammal to enhance transduction with said recombinant AAV vector ex vivo.

20. The method of claim 1, wherein said administering further comprises:

injecting said recombinant AAV vector into the portal vasculature of said mammal.

21. The method of claim 20, wherein said mammal is a human.

22. The method of claim 21, wherein said polynucleotide encodes a therapeutically effective polypeptide for treating a human disease or disorder.

23. The method of claim 21, wherein said polynucleotide comprises an antisense polynucleotide.

24. The method of claim 21, wherein said polynucleotide encodes a ribozyme.

25. The method of claim 1, wherein a polypeptide encoded by said polynucleotide is expressed and detectable in an elevated level in blood of the mammal as compared to a level of said polypeptide in blood of said mammal prior to said administering step.

26. The method of claim 1, wherein said liver cell is a hepatocyte.

(Amended)

27. A method of treating a liver disease or disorder in a mammal, comprising:

administering a therapeutically effective dosage of a recombinant adeno-associated [virus] (AAV) [vector] particles to the liver cells of said mammal, wherein said [recombinant AAV vector] rAAV particles comprise[s] a polynucleotide [which encodes a product with a therapeutic effect on ~~said~~ disease or disorder]

[... a therapeutic protein selected from the group consisting of ... and ...]

- 46 -

control of a regulatory region for gene expression in human liver cells, wherein the viral particles provide for liver-specific expression of said therapeutic protein or infection of said liver cells

28. The method of claim 27, wherein said polynucleotide encodes a therapeutically effective polypeptide for treating said liver disease or disorder.

29. The method of claim 27, wherein said polynucleotide encodes a ribozyme.

30. The method of claim 27, wherein said polynucleotide comprises an antisense polynucleotide.

(Amended)

31. A method of treating a disease or disorder in a mammal, comprising:

administering a therapeutically effective dosage of a recombinant adeno-associated (AAV) vector to the liver cells of said mammal, wherein said recombinant AAV vector comprises a polynucleotide operably linked to a liver-specific promoter or enhancer [that specifically functions in liver cells], wherein said polynucleotide encodes [a product with a therapeutic effect on said disease or disorder] factor VIII, factor IX or GM-CSF.

32. The method of claim 31, wherein said liver cells are hepatocytes.

33. The method of claim 31, wherein said promoter is obtained from a virus.

34. The method of claim 33, wherein said virus is murine leukemia virus.

35. The method of claim 34, wherein said virus is Moloney murine leukemia virus.

(Amended)

36. A method of gene therapy for a mammal, comprising:

administering a therapeutically effective dosage of a recombinant adeno-associated (AAV) vector to the liver cells of said mammal, wherein said recombinant AAV vector comprises a polynucleotide which encodes [a gene product with

a therapeutic effect in said mammal. factor VIII, factor IX or GM-CSF.

37. The method of claim 36, further comprising:

identifying a therapeutically effective polypeptide encoded by said polynucleotide and

constructing a recombinant AAV vector comprising said polynucleotide operably linked to a promoter and a polyadenylation sequence.

38. The method of claim 37, wherein said mammal is a human.

39. The method of claim 36, wherein said liver cells are hepatocytes.

40. The method of claim 37, wherein said promoter is obtained from a virus.

41. The method of claim 40, wherein said virus is murine leukemia virus.

42. The method of claim 41, wherein said virus is Moloney murine leukemia virus.

(Amended)

43. A pharmaceutical composition for treating a [human] liver disorder comprising:

[a]recombinant adeno-associated (AAV) [vector] particles comprising a polynucleotide operably linked to a [promoter and a polyadenylation sequence]; and encoding factor VIII, factor IX or GM-CSF

a pharmaceutically acceptable carrier.

44. The composition of claim 43, wherein said promoter is obtained from a virus.

regulatory region for gene expression in human liver cells

45. The composition of claim 44, wherein said virus is murine leukemia virus.

46. The composition of claim 45, wherein said virus is